Virtual Experiments in Marine Bioacoustics: Whales, Fish, and Anthropogenic Sound

Dr. Ted W. Cranford, Chief Scientist
Quantitative Morphology Consulting, Inc.
2674 Russmar Dr.
San Diego, CA 92123-7944
phone: (619) 226-7944 fax: (619) 226-7944 email: Ted.W.Cranford@gmail.com

Dr. Petr Krysl
Jacobs School of Engineering
University of California
San Diego, CA 92093

Mr. Carl R. Schilt
Bigleaf Science Services
P.O. Box 225
North Bonneville, WA 98639

Dr. Anthony D. Hawkins
Loughine Ltd
Kincraig, Blairs, Aberdeen, Scotland AB125YT

Award Number: N00014-09-1-0611

LONG-TERM GOALS

This programmatic effort has three long-term goals. The **first** is to simulate bioacoustic interactions within and near individual fish. We developed a methodology that combines x-ray CT scans with tissue elasticity measurements and finite-element modeling software (WHACR). This technique has provided significant insights and discoveries regarding toothed whale bioacoustics (Cranford et al., 2008b; Cranford et al., 2008c), and now, within a fish's head. The **second** long-term goal is to improve and refine our ability to measure tissue elasticity in samples by building a portable device to measure physical properties from tissue samples. The **third** and final goal is to validated the finite element models and add more functionality to the WHACR software.

OBJECTIVES

The **primary** objective is to examine simulations of the otolith organs to elucidate their motion patterns in response to acoustic stimuli from different directions and frequencies. The **secondary** objective is to standardize and improve our ability to measure tissue properties; primarily Bulk Modulus and sound speed in tissue samples. The **tertiary** objective is to refine the vibroacoustic toolkit (WHACR) and validate these finite element models.

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE **30 SEP 2011**	2. REPORT TYPE	3. DATES COVERED **00-00-2011 to 00-00-2011**

4. TITLE AND SUBTITLE **Virtual Experiments in Marine Bioacoustics: Whales, Fish, and Anthropogenic Sound**	5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Quantitative Morphology Consulting, Inc.,2674 Russmar Dr,San Diego,CA,92123-3422**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR’S ACRONYM(S) 11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

APPROACH

In teleost fishes there are three pairs of otoliths, dense masses or "stones" of calcium carbonate, each sitting upon a patch of hair cells, all contained within fluid filled sacs. We have generated high-resolution CT-scans of the White Seabass (*Atractoscion nobilis*) and segmented the otolith organs from the scanned volumes for image analysis.

Otolith organs may allow fish to analyze sound frequency and direction, but the mechanism remains elusive. It is necessary to account for the ability of fish to discriminate sounds of differing frequency (reviewed by Enger) (1973). Sand has suggested that the movement patterns of the otoliths may be frequency dependent, and that the parts of the macula which are stimulated may depend upon frequency (Sand, 1974). Further studies are necessary before this notion can be confirmed.

Fish can determination the direction of a sound source (see review by Sand and colleagues) (Sand and Bleckmann, 2007; Sand and Karlsen, 1986). One obstacle to understanding the mechanisms for these abilities is our lack of knowledge about the movements of the otoliths themselves. For example, do otoliths show simple translation back and forth along the axis of sound wave propagation or are the motions more complex? Horner (1980) suggested that the saccular otolith can only rock within the fluid-filled sacs and upon the macula because of constraints from proximal structures and fibrous attachments within the ear.

In order to determine the effect that otolith shape might have on otolith motion, we ran two sets of simulations using WHACR, our numerical analysis software. In the first simulations (Extracted Otoliths Simulations), we extracted the shapes of all six otoliths from high-resolution microCT scans of the White Seabass. Those "otoliths" were assigned uniform calcareous material properties, immersed in a simulated shear-soft jelly, and exposed to different stimulus frequencies and directions.

In the second set of simulations (Simplified Otolith Simulations), we compared the responses of simplified "otolith" shapes with the results of the Extracted Otoliths Simulations. In this second simulation, we used two simple shapes, a "spherical otolith" and a "hemispherical otolith."

One crucial step in building a finite element model is a process called *validation*. This process tests the veracity of the model by comparing virtual simulations to actual experimental results. We are currently generating experimental results that will be compared to the virtual model simulations.

Our first attempts to develop a device to measure tissue elasticity were unsuccessful. We are currently developing an alternative methodology, modifying a device known as the Linear Skin Rheometer (LSR) as the central component (Hess et al., 2006). We will also combine this device with a means to measure sound velocity in the tissue samples.

WORK COMPLETED

We have examined the two elementary models of "otoliths" (Extracted Otoliths Simulations & Simplified Otolith Simulations) and report that the otoliths rock in response to planar harmonic waves from different directions and stimulus frequencies. We have drafted two manuscripts that report on each set of simulations. These reports are now in the review and revision process.

Development of new tools for the vibroacoustic toolkit (WHACR) continues. The newest tool will provide the capacity to change the scale of element size during simulations within the model. This will allow us to reduce computational overhead for large, whole-animal data sets.

A procedure for extrapolating the scattered or total harmonic pressure in the near field using the Helmholtz integral was implemented in the vibroacoustic toolkit. This tool will allow us to calculate a reverse projection of the head-related transfer function (HRTF) in a fraction of the time required for the forward projection, saving time and computational overhead. We have tested this algorithm on the CT dataset from a live bottlenose dolphin (acquired from the US Navy Marine Mammal Program). We will also calculate the forward projection of the sound field for use in our model validation efforts. The calculated HRTF sound field will be compared to the psychoacoustic HRTF measured from live bottlenose dolphins. These measurements with live dolphins are currently underway in Paul Nachtigall's laboratory on Cocoanut Island, Hawaii.

Challenges and Solutions

Fish: Anatomically, it is important to know the precise position of the sensory maculae with respect to the otoliths. Discovering this anatomic relationship has been problematic because the otoliths are so hard that they cannot be effectively sectioned in their anatomic context. We have now identified a staining method that will allow us to enhance the soft tissues in the CT scans. This method looks very promising.

Validation: In order to complete the model validation process, we need to acquire a fresh bottlenose dolphin specimen from the stranding network at the University of North Carolina at Wilmington. They have agreed to send a fresh specimen when one becomes available. Once we have the specimen we can quickly move forward to scan the specimen and run the requisite simulations.

Elasticity: We have had to restart our efforts to build a device that can measure tissue elasticity from necropsy samples. This was a setback but we are currently working on a new trajectory to accomplish this part of the project. The LSR device will be built and tested by a former naval officer and advanced engineering graduate student as part of completing his dissertation research.

Computer: Running vibroacoustic simulations using finite element models that contain millions of elements is a computationally intensive undertaking. At the beginning of this project, we purchased an advanced computer that contains 48 CPU cores and 128 GB of memory to satisfy our computational needs. Recently, this computer experienced a problem and had to be sent back to the factory to be repaired. We eagerly await the return of this valuable piece of equipment so that we can continue our modeling work.

RESULTS

Extracted Otoliths Simulations

We collected high-resolution anatomic data from a few small (~21-cm total length) dead *Atractoscion nobilis* (Sciaenidae) from southern California by means of a micro-CT scanner. The scan data was used to extract high-fidelity representations of the otoliths and build a finite element model (FEM) by the methods and tools developed by Krysl et al. (Krysl et al., 2008) and Cranford et al. (Cranford et al., 2008a; Cranford et al., 2008c). This FEM model allowed us to investigate the dynamic response of fish otoliths to incident planar acoustic waves whose wavelengths are much longer than the dimensions of the otoliths. The otoliths are modeled as embedded in a shear-soft fluid-like jelly. The simplified

model does not currently include any other structures, such as the nearby cranial bones nor influences of the swimbladder. The model space was simulated with two different sinusoidal signals (200 and 400 Hz) from several different directions with respect to the fish.

Shear forces result from the relative motion between the otolith surfaces and the shear-soft jelly that surrounds them in the model space. The results show that the 400-Hz simulation produces greater shear values (due to larger displacements of the otoliths), particularly in the dorsoventral dimension, than does the 200-Hz signal of the same magnitude and direction. Similarly, changing the direction of the acoustic stimulus also produces altered patterns of shear forces acting upon the surfaces of the otoliths. These FEM simulations produced intriguing results, suggesting that frequency and direction may be encoded by the unique rocking motions of the otoliths. If this rocking behavior holds true for actual otoliths then we will have discovered a basic mechanism for hearing acuity in fish.

Simplified Otolith Simulations

The intriguing success of the Extracted Otolith Simulations catalyzed an investigation into whether the simplest "otolith" shapes would produce similar intriguing results. In essence, do "spherical" or "hemispherical" shapes (scatterers) produce angular oscillations due to torque in the presence of relatively long wavelength acoustic stimuli? These simple scatterers can be considered as "abstractions" of otoliths because they are without the distinctive boat-shape and sculpting found on actual otoliths, features which are often used as keys to species identification. In these Simplified Otoliths Simulations we considered a progressive planar harmonic wave in an acoustic fluid with selected mass density, speed of sound, and frequency.

The acoustic waves impinge upon a stiff homogeneous scatterer (the simplified "otolith") of arbitrary shape, whose characteristic dimensions are all much smaller than the wavelength of the incident acoustic wave. In the case of a "spherical otolith," uniform pressure across the symmetrical sphere in the acoustic fluid does *not* generate an accelerating torque on the scatterer. However, the case of the ***hemispherical*** scatterer is different. There is a dynamic torque experienced by the hemispherical scatterer. The numerical analysis indicates that the X and Z components of the torque are identically zero (these are the directions of the normal to the propagating sound-waves, and the direction of the axis of the symmetry of the scatterer). However, the hemispherical scatterer oscillates (rotationally) about the Y axis (in this direction is parallel to the propagating wave fronts). It rocks!

Furthermore, for other non-spherical, *asymmetrical*, shapes, the scattered pressure will generate a dynamic torque which will result in angular motion (rocking) in accordance with the specific parameters and initial conditions. This *principle* suggests that all such "asymmetric" shapes, like otoliths will experience rocking in the presence of relatively long wavelength progressive wave stimuli.

We have drafted and submitted a manuscript about these simplified synthetic scatterer results and it is currently in the process of revision, pending changes for calculations of flow characteristics.

Anatomy of Otolith Organs and Sensory Maculae

The anatomic relationships between the otoliths and the sensory maculae are the basis for the input to the fish central nervous system. Discovering these relationships has been an intractable problem

caused by the hardness of the otoliths near the delicate nervous tissue. Fortunately, we have found a pre-scan Iodine staining technique developed by Metscher and colleagues (Metscher, 2009a; b) that will make these relationships visible after CT scanning.

In addition, we have salvaged the sensory maculae from five White Seabass specimens so that we can obtain a map of the hair cell directions. The hair cell maps are currently being produced in the laboratory of Dr. Art Popper in Maryland. The fine scale motions of the otoliths combined with the hair cell maps may provide clues to the means by which fish can discriminate the frequency and direction of a sound source.

Model Validation and Head-related Transfer Function

The experimental design for the HRTF experiments with live dolphins in Hawaii has been finalized and data collection has begun. That portion of the study should be complete by the end of the year.

The construction of the model that we will use for comparative purposes has started with the CT dataset from the live bottlenose dolphin acquired from the US Navy Marine Mammal Program. We will use this dataset until we obtain a fresh postmortem specimen from UNCW and can generate a higher resolution model.

IMPACT/APPLICATION

The Extracted Otoliths Simulations and the Simplified Otolith Simulations produced similar "rocking" motions from unsymmetrical "otoliths." This consistency hints at a potential principle of otolith motion that might be common to all teleost fish. Future iterations will include additional anatomic components within the model.

RELATED PROJECTS

This project is an outgrowth of the methodology we have developed over the past eight years (Cranford et al., 2010; Krysl et al., 2008; Krysl et al., 2006; Soldevilla et al., 2005). We are currently using the same basic methods to study interaction between toothed whale anatomy and selected sounds.

PUBLICATIONS

Krysl P, Kagey H. (In press). Reformulation of Nodally Integrated Continuum Elements (NICE) to attain insensitivity to distortion. Int. J. Numer. Meth. Engng.

Krysl P, Monterrubio L. (2011). Modelling the proportional far-field pressure using the WHACR software. Report to University of California. San Diego. p 9.

Krysl P, Trijoulet V, Cranford TW. (In press). Validation of a vibroacoustic finite-element model using bottlenose dolphin experiments. In: Popper AN, Hawkins AD, editors. Effects of Noise on Aquatic Life. New York: Springer Science+Business Media, LLC.

Krysl P, Trijoulet V, Cranford TW. (In review). Focusing the dolphin biosonar beam in stages: Validation of a vibroacoustic finite element model using bottlenose dolphin simulations. Aquatic Mammals.

Schilt CR, Cranford TW, Krysl P, Shadwick RE, Hawkins AD. (In press). Vibration of the otoliths in a teleost. In: Popper AN, Hawkins AD, editors. Effects of Noise on Aquatic Life. New York: Springer Science+Business Media, LLC.

Schilt CR, Cranford TW, Krysl P., Hawkins AD. (In prep). A finite-element modeling study suggests that different sound stimulus frequencies and directions may produce different motions of a sciaenid fish's otoliths. Manuscript for Hearing Research.

Krysl P., Hawkins AD., Schilt CR, Cranford T.W. (In review). Rocking of rigid scatterers generated by progressive planar acoustic waves.

REFERENCES

Cranford TW, Krysl P, Amundin M. 2010. A new acoustic portal into the odontocete ear and vibrational analysis of the tympanoperiotic complex. PLoS ONE: Public Library of Science. p 1-29.

Cranford TW, Krysl P, Hildebrand J. 2008a. Sound pathways revealed: Simulated sound transmission and reception in Cuvier's beaked whale (*Ziphius cavirostris*) using the vibro-acoustic toolkit. Bioacoustics 17(3):68-70.

Cranford TW, Krysl P, Hildebrand JA. 2008b. Acoustic pathways revealed: Simulated sound transmission and reception in Cuvier's beaked whale (*Ziphius cavirostris*). Bioinspiration & Biomimetics 3:1-10.

Cranford TW, McKenna MF, Soldevilla MS, Wiggins SM, Shadwick RE, Goldbogen J, Krysl P, St. Leger JA, Hildebrand JA. 2008c. Anatomic geometry of sound transmission and reception in Cuvier's beaked whale (*Ziphius cavirostris*). Anat Rec 291(4):353-378.

Enger PS, Hawkins AD, Sand O, C.J. C. 1973. Directional sensitivity of saccular microphonic potentials in the haddock. The Journal of Experimental Biology 59:425-433.

Hess MM, Mueller F, Kobler JB, Zeitels SM, Goodyer E. 2006. Measurements of vocal fold elasticity using the linear skin rheometer. Folia Phoniatrica et Logopaedica 58:207-216.

Horner K. 1980. Structure and function of the codfish ear [Doctoral Dissertation]. Aberdeen: University of Aberdeen.

Krysl P, Cranford TW, Hildebrand JA. 2008. Lagrangian finite element treatment of transient vibration/acoustics of biosolids immersed in fluids. Int J Numer Meth Engng 74(5):754-775.

Krysl P, Cranford TW, Wiggins SM, Hildebrand JA. 2006. Simulating the effect of high-intensity sound on cetaceans: Modeling approach and a case study for Cuvier's beaked whale (*Ziphius cavirostris*). The Journal of the Acoustical Society of America 120(4):2328-2339.

Metscher BD. 2009a. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. BMC Physiology 9(11):1-14.

Metscher BD. 2009b. MicroCT for developmental biology: A versatile tool for high-contrast 3d imaging at histological resolution. Dev Dyn 238:632- 640.

Sand O. 1974. Directional sensitivity of microphonic potentials from the perch ear. The Journal of Experimental Biology 60:881-899.

Sand O, Bleckmann H. 2007. Orientation to auditory and lateral line stimuli. In: Webb JF, Fay RR, Popper AN, editors. Fish Bioacoustics. New York: Springer-Verlag. p 183-232.

Sand O, Karlsen HE. 1986. Detection of infrasound by the Atlantic cod. The Journal of Experimental Biology 125:197-204.

Soldevilla MS, McKenna MF, Wiggins SM, Shadwick RE, Cranford TW, Hildebrand JA. 2005. Cuvier's beaked whale (*Ziphius cavirostris*) head tissues: physical properties and CT imaging. The Journal of Experimental Biology 208:2319-2332.